

POTENTIAL HAZARDS OF AN INORGANIC FERTILIZER (WEATFERT) FOR THE BROWN GARDEN SNAIL (*EOBANIA VERMICULATA* MÜLLER, 1774): GROWTH, HISTOLOGICAL AND BIOCHEMICAL CHANGES AND BIOMARKERS

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Abstract. Land snails are often used to monitor soil pollution. The present study aimed to investigate the effects of Weatfert R complex mineral nitrogen, phosphorus, and potassium (N: P: K) fertilizer with high phosphorous content (NPK: 8/36/15 + SO₃:13) on *Eobania* (syn. *Helix*) *vermiculata* used as a bioindicator species. Moreover, the histological and biochemical changes in the digestive gland were examined and biomarkers of environmental stress (glutathione peroxidase and lactate dehydrogenase activity) measured. Results showed that the fertilizer tested has a negative impact on the growth rate of juvenile snails. They also revealed alterations in the structure of the digestive gland and changes in biomarker response values in the field, accompanied by significant correlations with bioconcentration. Meanwhile, the biochemical parameters showed varying results representing an increase in the protein rate and a decrease in lipid and carbohydrate rates after treatment with weatfertin. Overall, our study showed that the fertilizer tested could have potential hazards on *E. vermiculata* and this snail was a suitable sentinel organism and the selected biomarkers are efficient for soil assessment.

Keywords: chemical fertilizers, bioindicator organism, growth rate, digestive gland, biochemical changes, biomarkers of oxidative stress

Introduction

Increasing food production is the main concern of all countries, as world population is expected to grow to nearly 10 billion by 2050 (Gill and Garg, 1998). It has led to the application of a wide variety of chemicals, pesticides and inorganic fertilizers to agricultural land (Yahiaabadi et al., 2018). The usage of inorganic fertilizers has grown in various parts of the world (Lalthanzra and Ramanujam, 2010) and the impact of intensive use is seen not only in terms of the soil quality but also on the survival of soil organisms dwelling there in (Rai et al., 2014). In literature, some researchers have shown that chemical fertilizers are harmful for soil organisms, but on the contrary, others have found that they are beneficial for their food supply (Rai et al., 2014).

Soil is home to different life forms and a reservoir of all the agrochemicals (Yahiaabadi et al., 2018). To test the effects of chemicals on soil organisms, several biological methods have been applied to nematodes, earthworms, collembolans, and snails (Druart et al., 2011). Mollusca are major part of the world fauna, and the second-largest phylum of the animal kingdom. The terrestrial Gastropoda is one of the most diverse groups of animals, both in shape and habit (Sallam and El-Wakeil, 2012). These animals are of great importance not only due

to their food value but also for their use in road construction, lime production, preparation of feed for poultry, and finally they indirectly contribute to organic matter decomposition (Bhowmik, 2005).

Several studies have been carried out to determine their activity, especially fertilizers that reach water streams during agricultural activities and may kill snails (Ragab and Showrky, 2006; Ismail, 2009) or make their environmental conditions unsuitable for their life (El-Deeb, 2007). The toxicity endpoints of most of the bioassays are survival, growth, and reproduction (Roh et al., 2010).

Land snails have also been widely used as a sentinel species for the assessment of pollution in terrestrial ecosystems (Douafer et al., 2020). They accumulate various contaminants in their soft tissues, especially the digestive gland, as well as they are helpful species in monitoring the exposure to trace metals, agrochemicals, urban pollution and electromagnetic agents (Regoli et al., 2006). The impact on cellular and biochemical alterations of digestive glands as biomarkers of toxicants have been investigated (Suyman et al., 2006).

Eobania vermiculata (Müller, 1774), the brown garden snail, belongs to the family of Helicidae and is distributed worldwide especially in the Mediterranean area (Radwan et al., 2008). Commonly used as test organism in ecotoxicology studies, it presents several advantages, since it is relatively easy to adapt and maintain in the laboratory conditions, and its physiology is well known (Yousef, 2011).

Terrestrial snails are well known for their capacities to accumulate different classes of chemicals in their tissues, particularly, the hepatopancreas (digestive gland) (Regoli et al., 2006). The possible use of cellular alterations on the gastropods' hepato-pancreas as biomarkers for the exposure to xenobiotics have been investigated (Radwan et al., 2008). The digestive gland is the key organ of chemicals detoxification.

The biochemical responses in organisms exposed to toxic contaminants have been used as biomarkers (Abd-El Azeem and Sheir, 2018). These biomarkers measure the interaction between a biological system and an environmental agent, which might be chemical, physical, or biological (WHO, 1993). In vivo, their inhibition or their induction is a good environmental tool to assess the exposure and the effects of xenobiotics on organisms (Porte et al., 2005).

The use of oxidative stress biomarkers is of potential interest for the assessment of the pollutants impact (Versclar et al., 2008). Moreover, the interaction between xenobiotics and the components of the antioxidant defense systems play an important role in the ecotoxicological response of an organism to its environment (Regoli et al., 2006).

This study was designed to measure the effects during a chronic exposure (3 months) of an inorganic fertilizer with high phosphorus content (Weatfert^R) on survival and growth rates of *E. vermiculata*. The main biochemical components (proteins, carbohydrates and lipids) and the biomarker responses biomarkers were also determined in the digestive gland of the snails. In addition, the histological alterations of the digestive gland due to fertilizer accumulation were examined.

Materials and methods

Snail's collection and maintenance

E. vermiculata was collected from untreated area in Bekkaria (35° 22' 20" N; 8° 14' 32" E) (Tebessa, Northeast Algeria) in autumn and winter of 2017-2018. The collection

was always made manually. The collected snails (1.90 ± 0.029 mm in the shell diameter and 3.43 ± 0.04 g in body weight) were transferred in glass boxes ($20 \times 20 \times 20$ cm) with 5 cm layer of moistened soil at the bottom. They were identified according to the key of (Godan, 1983). The animals were fed with lettuce leaves and the food was renewed on alternate days. The snails were acclimatized for 2 weeks under laboratory conditions (16 ± 1 °C; 31%) relative humidity and a 18:6 h light-dark cycle before the beginning of experiments (Gomot, 1994).

Chemicals and treatment

Weatfert (Profert, Bejaia, Algeria) is a trade name of an inorganic fertilizer. It contains a mixture of the three principal nutrients (8% N + 36% P₂O₅ + 15% K₂O) + 13%SO₃. Thereafter, 16 snails were added to each box and the sets were subdivided as follows after 14 days of adaptation; three boxes were applied for a control test. Six boxes were treated with two doses of Weatfert, (i) the first treatment with a recommended agricultural dose ($D_1 = 500$ mg/400 cm²) normally used by farmers (1.5 quintals per hectare) and (ii) the second treatment with a recommended agricultural dose x2 ($D_2 = 1000$ mg/400 cm²). The amount of fertilizer required was determined by the total area of the experimental box (400 cm²). These were added to the soil surface and then mixed thoroughly with enough water to ensure a homogeneous mixture. For the control treatment, distilled water was used. Treatment was done for 3 months. The tested fertilizer was replaced weekly using the same concentration.

Determination of growth inhibition

Weight growth is assessed weekly by weighing the snails with an analytical balance (Ohaus® Analytical, Switzerland). The inhibition percentage of average weight (W_{ip}) (ISO 15952) (Eq. 1) is calculated to compare the average weight of the groups treated with that of the control group.

$$W_{ip} \text{ of group } G_x = \frac{(W_{Tn} - W_{T0}) - (W_{Gn} - W_{G0})}{(W_{Tn} - W_{T0})} \times 100 \quad (\text{Eq.1})$$

where:

W_{ip} inhibition percentage of average weight

Group G_x represents the groups of treated snails G_1 , G_2

W_{Tn} is the mass of the snails in the control group at time $t = n$ weeks

W_{T0} is the mass of control group snails at the start of the experiment

W_{Gn} is the mass of snails in the G_x group at time $t = n$ weeks

W_{G0} is the mass of G_x group snails at the start of experience

Biochemical composition of digestive glands

Digestive glands from control and treated snails were dissected out at different times (0, 1, 2 and 3 months). The main biochemical components (proteins, carbohydrates and lipids) were extracted following the procedure of Shibko et al. (1966). Fragments (100 mg) of the digestive gland were extracted in 1 mL of TCA (20%). In brief, quantification of proteins was carried following the Coomassie Brilliant Blue G-250 dye-binding method of Bradford (1976) with bovine serum albumin as a standard. The absorbance was measured at 595 nm. Carbohydrates were determined as described by Duchateau and Florkin (1959) using anthrone as reagent

and glucose as standard. Lipids were measured by the vanillin method of Goldsworthy et al. (1972). Data were expressed in μg per mg of fresh tissue and assays conducted with three replicates per treatment each containing 100 mg of digestive gland fragment.

Biomarker assays

Two doses (500 and 1000 mg/400 cm²) of Weatfert were orally applied on *E. vermiculata* and its effects examined on LDH and GPx activities measured at various times (0, 1, 2 and 3 months during treatment. GPx activity was determined according to the method of Flohe and Gunzler (1984). Fragments of digestive gland (100-200 mg) were homogenized in 1 ml of phosphate buffer (pH 7.8). The homogenate was centrifuged (3000 rpm for 10 min) and then the supernatant recovered for use as enzyme source. The assay was performed with 200 μl of supernatant added to 400 μl of GSH solution (0.2 mM, pH 10). Absorbance reading was done after 5 min at 412 nm. The LDH assay was based on the conversion of lactate to pyruvate or pyruvate to lactate. The lactate dehydrogenase activity (LDH) was spectrophotometrically measured according to the method of Hill and Lévi (1954) as previously described (Sifi and Soltani, 2019). It uses NAD (nicotinamide adenine dinucleotide) as substrate. Fragments of digestive gland (100-200 mg) were homogenized in 1 ml of Tris/HCl (0.1 M, pH 7.2). The homogenate was centrifuged (3000 rpm for 5 min) and then the supernatant recovered for use as enzyme source. The assay was performed with 50 μl of supernatant added to 675 μl of substrate buffer (0.2 M, pH 10) and 50 μl of NAD solution. The absorbance reading was done every minute for 5 min at 340 nm. The protein content was evaluated according to Bradford (1976) using bovine serum albumin as standard (BSA, Sigma). The activity was expressed as $\mu\text{M}/\text{min}/\text{mg}$ protein.

Histological procedure

The histological procedure was performed according to the method of Gabe (1968). In brief, 16 snails from each control and treated series (D₁ and D₂) were randomly collected (at two periods: 0 day and 3 months) and the digestive glands were dissected. Each whole digestive gland was fixed in formalin solutions (30%) for 24 h, and dehydrated in baths of alcohol with increasing concentrations (70, 80, 90 and 95%). Then, specimens were cleared in xylene and embedded in paraffin wax. Serial sections (4 μm) were prepared by a Leitz microtome and stained using the hematoxylin and eosin. Sections were then mounted and covered with glass cover. Histological sections were examined under the light microscope (Leica DM LB2).

Statistics

Data are presented as a mean value \pm SEM (standard error mean) in each treatment group. The normality of data was verified using the Kolmogorov-Smirnov test, and the homogeneity of variances was checked by Levene's test. Comparison of the experimental groups was tested by analysis of variance (ANOVA), and means were tested for statistical significance by a post hoc Tukey's honestly significant difference test. The statistical tests were performed using GraphPad Prism, version 7.00 (GraphPad Software, San Diego, CA, USA), where $p < 0.05$ indicates a statistically significant difference.

Results and discussion

Effects on growth of snails

The growth of juvenile snails is noted monthly during the treatment period (3 months). The tested fertilizer resulted in an increase of growth inhibition (%) of snails without dose-response relationship and a decrease during the tested time. The growth of these snails is inhibited after treatment with two doses respectively, and displays percentages ranging from 69 and 78% for the first month, from 55 to 61% for the second month and from 49 to 54% for the third month (Fig. 1).

The use of change in body mass as a biomarker is ecologically relevant, as high body mass losses are believed to have negative effects on survival and reproduction (Dittbrenner, 2010). Treatments with three types of NPK fertilizers (high nitrogen, high phosphorus and balanced) (El-Deeb, 2017) or with urea product (Ragab and Shoukry, 2006) were found to influence snail growth. Meanwhile, Abdel Hamid et al. (1998) reported that urea and ammonium nitrate reduced the growth of juvenile *B. alexandrina* snails. Schuytema et al. (1994) reported a significant reduction in weight and shell diameter of *Helix aspersa* snails treated with Aminocarb, Methyl parathion and Paraquat; the responses observed varied according to the type of pesticides and dose administered. Furthermore, the overall results seem to indicate that the reduction in growth is linked to the mode (Sallama et al., 2005; Radwan et al., 2008) and to the duration of treatment exposure (Coeurdassier et al., 1957). Similarly, growth of snails (*Helix aspersa*) exposed to pesticides (thiamethoxam and tefluthrin) was affected (Ait-Hamlet, 2012). Afomezie et al. (2011) found that NPK soil treatment affects snail's performance by reducing their final weight and weight gain. Bhattacharya and Sahu (2016) showed clearly that the recommended dose of NPK did not cause earthworm mortality.

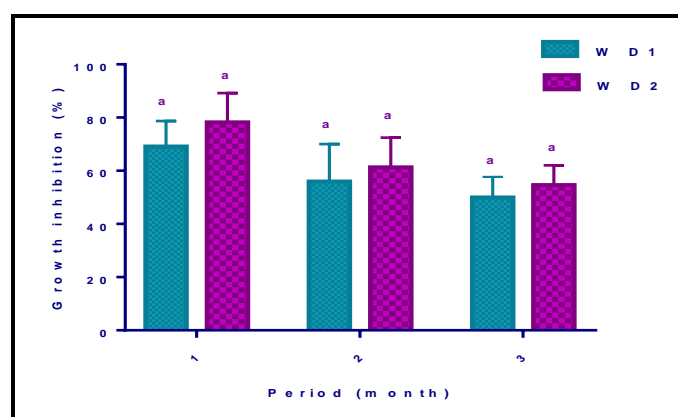


Figure 1. Effects of fertilizer administrated at two doses on growth inhibition (%) in juvenile snails of *E. vermiculata* (mean ± SEM, $n = 5$ repeats, each containing 10 individuals): Same lowercase letters indicate no-significant differences at the same time based on *T* student test ($p > 0.05$)

Effect on biochemical composition of digestive glands

The fertilizer Weatfert was applied to juveniles of *E. vermiculata* during 3 months. Its effects were evaluated on the main biochemical components (carbohydrates, lipids,

proteins) of digestive glands at different times (0, 1, 2 and 3 months) after treatment (Table 1).

Results of the protein amounts showed a significant increase in treated series with the highest dose (dose 2) at the first ($p = 0.007$) and the third month ($p = 0.008$) as compared to control series. At the second month, the two applied doses induced a significant increase (control vs dose 1: $p = 0.032$; control vs dose 2: $p = 0.013$) of this component, without dose-response relationship.

Table 1. Effects of Weatfert on protein, lipid and carbohydrates rates in digestive gland ($\mu\text{g}/\text{mg}$ of fresh tissue; $n = 3$ pools each containing 100 mg of fresh tissue) at different periods during treatment in the juveniles of *E. vermiculata* (mean \pm SEM)

Components	Times (month)	Control	Dose 1	Dose 2
Proteins	0	57.73 \pm 3.27a, A	56.07 \pm 3.27a, A	65.78 \pm 4.19a, A
	1	59.25 \pm 3.55a, A	72.91 \pm 5.00a, B	88.64 \pm 7.12b, B
	2	69.25 \pm 2.48a, A	87.46 \pm 5.61b, C	91.57 \pm 5.84b, B
	3	89.96 \pm 11.31a, B	97.25 \pm 8.20a, C	130.66 \pm 1.85b, C
Lipids	0	36.69 \pm 3.31a, A	36.33 \pm 1.17a, A	36.11 \pm 3.16a, A
	1	38.28 \pm 1.77a, A	27.88 \pm 1.24b, B	19.84 \pm 2.46c, B
	2	39.61 \pm 1.49a, A	27.00 \pm 2.57b, B	19.39 \pm 1.32c, B
	3	57.73 \pm 1.78a, B	18.57 \pm 2.28b, C	14.65 \pm 0.70b, B
Carbohydrates	0	61.28 \pm 1.49a, A	63.40 \pm 1.12a, A	63.81 \pm 1.83a, A
	1	61.74 \pm 1.88 a, A	59.31 \pm 2.29a, A	52.61 \pm 3.57a, B
	2	64.02 \pm 1.79a, A	57.92 \pm 0.89a, A	40.81 \pm 3.41b, C
	3	74.28 \pm 0.60a, B	48.94 \pm 4.49b, B	22.92 \pm 1.18c, D

Mean values followed by different lowercase letter indicate significant differences between control and treated series

Mean values followed by different capital letter indicate significant differences between periods based on Tukey's HSD test at $p < 0.05$

Concerning the lipid rate, a significant reduction was observed in the first (control vs dose 1: $p = 0.005$; control vs dose 2: $p < 0.001$; dose 1 vs dose 2: $p = 0.0184$), the second (control vs dose 1: $p = 0.0019$; control vs dose 2: < 0.001 ; dose 1 vs dose 2: $p = 0.0218$) and the third month (control vs dose 1: $p < 0.001$; control vs dose 2: $p < 0.001$) in the treated series with the two tested doses compared to controls.

Finally, our results also revealed a significant decrease in carbohydrates amounts with the highest dose at the first month (control vs dose 2: $p = 0.0496$), the second (control vs dose 2: $p = 0.0002$; dose 1 vs dose 2: $p = 0.001$), and the third month (control vs dose 1: $p = 0.0003$; control vs dose 2: $p < 0.0001$; dose 1 vs dose 2: $p = 0.0003$) as compared to control series (Table 1).

Biochemical and enzymatic parameters in organisms exposed to toxic contaminants have been used as biomarkers and may be considered an important diagnostic tool to assess the exposure and effects of xenobiotics (McClouglin et al., 2008). The increasing demands of organisms to energy during stress to detoxify, bio-transform and excrete the toxicants is achieved by the use of carbohydrate as the principal and immediate energy source (Umminger, 1977).

Glycogen breakdown and increased glucose are among the well-known mechanisms to provide energy in mollusks in stressful environments (Tunholi et al., 2017). The energy is used to cope with the cytotoxicity induced by pesticides. In our research, the tested fertilizer, weatfert caused a significant reduction in the energy reserves as evidenced by a reduction in carbohydrate rates. In this regard reported a decrease in tissue glycogen levels and an increase in hemolymph glucose concentration in the freshwater snails (*Bulinus truncates*) exposed to glyphosate herbicide (Bakry et al., 2012). Similarly, the reduction of tissue glycogen has been reported in the mud snails (*Amphibola crenata*) exposed to waterborne cadmium (De Silva et al., 2018). The results of Mohammadein et al. (2013) and Radwan et al. (2008) disagree with our results, who found a significant decrease in the glycogen content of the digestive gland in land snails treated with heavy metal and chemicals (methomyl and methiocarb), respectively. This may result from an increased rate of glycogen breakdown (glycogenolysis). Moreover the decrease in glycogen content may have an indirect effect on the protein and lipid reserves in *E. vermiculata*. The results of Banaee and Taheri (2019) indicated significantly lower levels of glycogen and total antioxidant in the cells in the freshwater snail, *Galba truncatula* exposed to sewage compared to controls.

Proteins are one of the major groups of biological materials comprising the chief nitrogenous elements of the body tissues. The concentration of free amino acids in mollusk varies with pollution levels (Bishop, 1983). Proteins are mainly involved in the architecture of the cell (Radwan et al., 2008). During chronic periods of stress, the snails needed more energy to detoxify the toxicants and to overcome the induced stress (El-Shenawy et al., 2012) especially when they have a limited amount of carbohydrates and lipids (Radwan et al., 2008). Our experiment showed a significant increase in protein rates in *E. vermiculata* treated with Weatfert fertilizer. The results of the study of Khalil (2016) suggest that the exposure to sublethal doses of pesticide, methomyl-lannate may influence total protein metabolism. Previously, Bakry et al. (2011) reported that two pesticides, deltamethrin and malathion had qualitative and quantitative effect on the protein patterns of *Helisoma duryi* snails. Furthermore, the electrophoretic profile of total proteins from *Biomphalaria alexandrina* snails treated with diazinon and profenfos showed less number of protein bands, indicating that the pesticides were thought to induce damage in these snails (Bakry et al., 2013). Padmaja and Rao (1994) suggested that the decrease in snail proteins treated with pesticides could be due to several mechanisms namely (a) the formation of lipoproteins which are utilized for the repair of damaged cells and tissue organelles, and (b) the direct utilization by cells for energy requirements (Radwan et al., 2008).

Lipids play extremely important roles in the normal function of a cell. They not only serve as highly reduced storage form of energy, but they also play an intimate role in the structure of cell membranes and the organelles found in the cell (Kandil et al., 2009). Indeed, lipids are the preferred energy fuel offered to tissues when needed after carbohydrates. During periods of chronic stress, they also constitute another source of energy (Moussard, 1999). When snails have a limited amount of carbohydrates and fat, the alternative energy source to meet the increased demand for energy is protein (Moussard, 1999). A decrease in total lipids was noted in *E. vermiculata* snails treated with Weatfert fertilizer compared to control snails. In addition, these results are similar to those of Ait Hamlet et al. (2012) showed that the Thiamethoxam causes a disturbance in the lipids content in the digestive gland of treated snails compared with controls.

Moreover, depletion of some long chain and short chain fatty acids may be explained on the basis that reduction in rates of glucose metabolism in the snails was balanced through the stimulation of triglyceride hydrolysis and fatty acid oxidation (Ait-Hamlet et al., 2012).

Effects on biomarkers responses

Results of the specific activities of antioxidant defense enzyme (GPx and LDH) in *E. vermiculata* exposed to two doses of fertilizer are shown in Figures 2 and 3. The results showed a significant increase in GPx activity at the first ($p < 0.001$), the second ($p < 0.001$) and the third month ($p < 0.001$) respectively as compared to control series, with dose-response relationship at the first (dose 1 vs dose 2: $p = 0.016$) and the third month (dose 1 vs dose 2: $p = 0.006$) (Fig. 2).

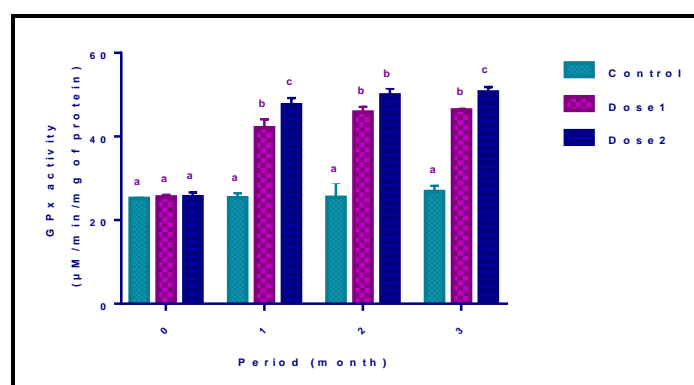


Figure 2. Effect of Weatfert on LDH activities ($\mu\text{M}/\text{min}/\text{mg}$ of proteins) in the juvenile snails of *E. vermiculata* at different times during treatment (mean \pm SEM, $n = 3$). The different lowercase letters indicate significant differences at the same time based on Tukey's HSD test ($p < 0.05$)

Finally, our results also showed a significant increase in the activity of LDH in the treated series (dose 1 and dose 2) at all tested periods: the first (control vs dose 1: $p = 0.0031$; control vs dose 2: $p < 0.001$), the second (control vs dose 1: $p = 0.0015$; control vs dose 2: $p < 0.001$) and the third month ($p < 0.001$) as compared to control series respectively (Fig. 3). In addition, we note a significant difference between the two tested doses only at the first month (dose 1 vs dose 2: $p = 0.036$).

Biomarkers are essential for ecosystem health assessment and management (Boyd, 2010). The study of the antioxidant defense system is increased because of its potential utility to provide biochemical biomarkers that can be used in environmental monitoring systems (Ballesteros, 2009).

Glutathione peroxidase (GPx) known as the most important peroxidase ensures the detoxification of peroxide and hydroperoxides to water and hydroxyl compounds, respectively (Pinto et al., 2003) so it play a protective role against oxidative stress (Van Der Oost et al., 1998; Van Der Oost et al., 2003).

Our results show a significant increase in GPx activity was found after both the first and second weeks in snails exposed to Weatfert fertilizer. These are in agreement with those of Farid et al. (2009) which reveal an increase of GPx in *Lymnaea natalensis* after treatment with niclosamide. Similar observations have been reported by El-Shennawy et al. (2012) who observed elevated GPx levels in snails exposed to cadmium, lead, copper

and iron. This increase may be attributed to free radical production (Hermes et al., 2020) as shown by Orbea et al. (2000) and Radwan et al. (2010) on other snail species exposed to other pollutants. In contrast, a slight inhibition was observed in GPx activity with high phosphorus fertilizer treatment in *Biomphalaria alexandrina* snails (El-Deeb, 2017).

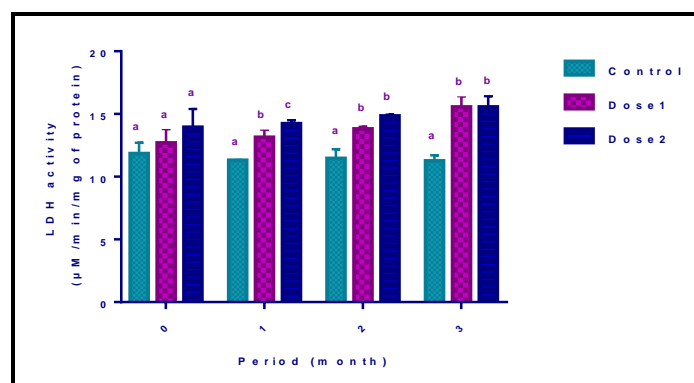


Figure 3. Effect of Weatfert on GPx activities ($\mu\text{M}/\text{min}/\text{mg}$ of proteins) in the juvenile snails of *E. vermiculata* at different times during treatment (mean \pm SEM, $n = 3$). The different lowercase letters indicate significant differences at the same time based on Tukey's HSD test ($p < 0.05$)

Lactate dehydrogenase (LDH) is an enzyme found in nearly all living cells (animals, plants and prokaryotes). Lactate dehydrogenase converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply and it performs the reverse reaction during the Cori cycle in the liver. Our results are in accordance with Banaee et al. (2019) found that there was a significant increase in the activity of LDH in *G. truncatula* exposed to cadmium and dimethoate. A reduction was shown in LDH activity in *B. alexandrina* treated with atrazine and Roundup (Bakey et al., 2012). In the study of Banaee and Taheri (2019) a significant increase was observed in the activity of LDH in the *G. truncatula* exposed to sewage, which could be a physiological response to the stressful environmental. Increased LDH activity in the soft tissues of snails could be attributed to hypoxia and increased anaerobic glycolysis. This enzyme is involved in the metabolism of carbohydrates in cells and plays a key role in maintaining the balance between the catabolism and anabolism of carbohydrates in mollusks (Chen et al., 2011). In a stressful environment, LDH converts pyruvate into lactate, which in turn leads to the enhanced concentration of carboxylic acid (lactic acid) in tissues and hemolymph. Therefore, the pH of tissues and hemolymph decreases and causes specific physiological changes (Tunholi et al., 2017). In a study by Abdel-Halim et al. (2013), the LDH activity was observed to increase, which indicate a physiological response to the hypoxia-induced stress following the exposure of *Helix aspersa* to heavy metals. In addition, a significant increase has been reported in the LDH activity in the land snails (*E. vermiculata*) exposed to methomyl (Khalil, 2016). A significant increase in the activity of LDH was observed in *Deroceras reticulatum* exposed to Caselio fertilizer (Abd-El Azeem and Sheir, 2018). LDH was chosen as a biomarker of the oxidative stress induced by the tested material (fertilizer, PMR). Increased oxidative stress biomarkers as lipid peroxidase in fertilizers polluted areas was documented by Yousef et al. (2017). Salama et al. (2005) recorded significant increase of the level of LDH in

the land snail, *Helix aspersa* after exposure to several chemicals such as methomyl, carbofuran and chlorpyrifos. The increased release of LDH in mantle tissue is an indicative of cellular or membrane damage (Abd-El Azeem and Sheir, 2018).

Effect on digestive gland structure

Figure 4A, B and C represents the structure of digestive glands in control and treated snails after 3 months of exposition.

In control snails, the digestive gland epithelium presented a normal appearance at 0 day (Fig. 4A) and after 3 months (Fig. 4B) experimentation. The cells were readily distinguished, together with less abundant excretory and calcium cells.

In treated series, the examination of histological structure shows modifications. As shown in Figure 4C and D, the epithelium of snails treated presented a significant hyperplasia of the gland, with the presence of fat cells. In addition, no necrosis and cell congestion were observed.

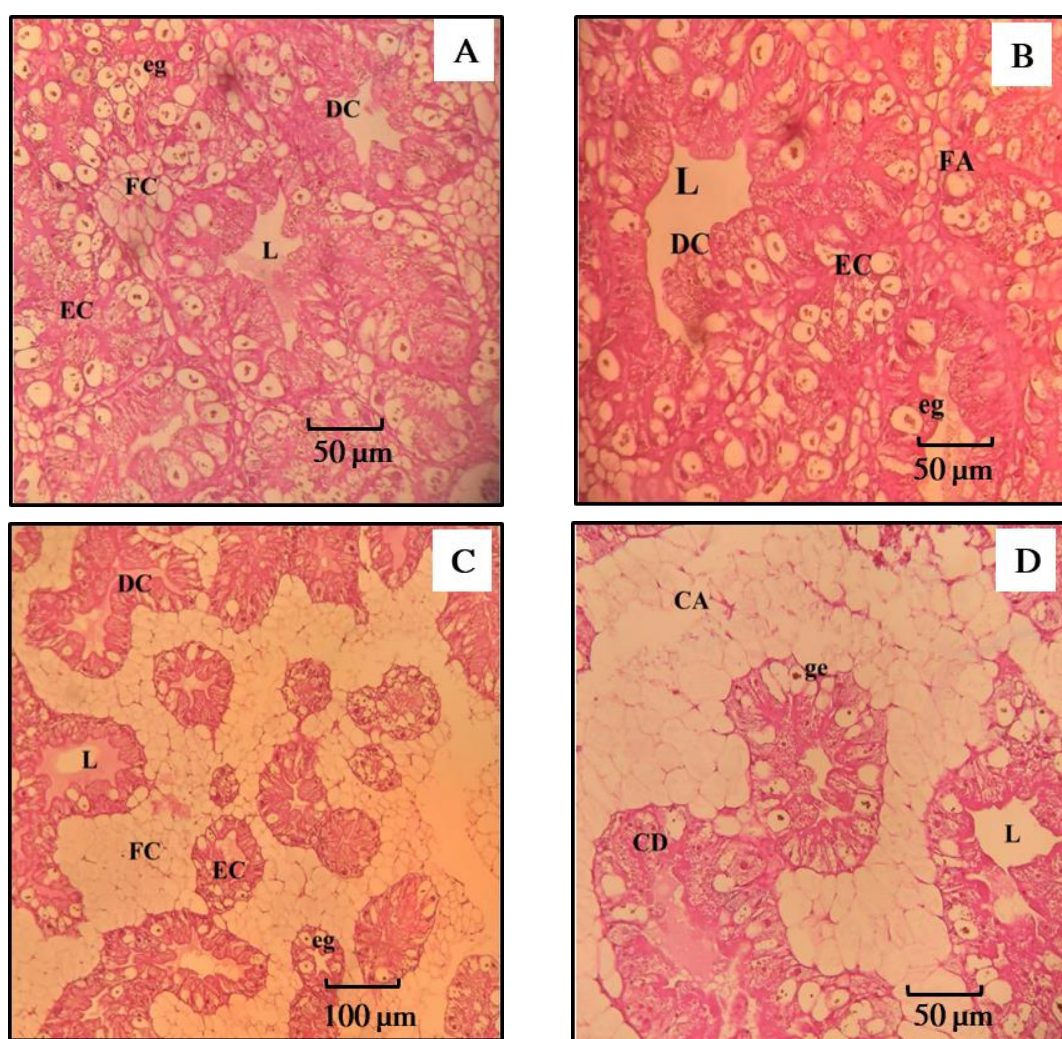


Figure 4. Transverse sections of digestive glands: in control snails (0 day) (A), in control snails (3 months) (B), in treated snails (D1) (3 months) (C) and in treated snails (D2) (3 months) (D). Calcium Cell (CA); Digestive cell (DC); Excretory cell (EC); Excretory granules (eg); Fatty cell (FC); Tubular lumen (L)

Beside the role of fertilizers in the growth of the plant it has a molluscidal effect. Sheir (2015) found that Caselio (plant fertilizer) caused tissue damage in the digestive tubules as necrosis, fusion and increased lipofuscin pigment in the freshwater snail, *Lanistes carinatus*. El-Deeb et al. (2015) exposed *Biomphalaria alexandrina* snails to the inorganic fertilizers (high phosphorus and high nitrogen content) and recorded histological alterations in the digestive glands. Tribskorn and Ebert (1989) studied the effect of carbamate and metaldehyde molluscicides on the digestive tract of *Deroceras reticulatum*. He described that mucus deficiency in the digestive tract due to metaldehyde exposure. Dummee et al. (2012) exposed the golden apple snail, *Pomacea canaliculata* to contaminated sediment with the metals (Fe, Mn, Cu and Zn) for two months. The results showed metals accumulation in the digestive tract and digestive gland higher than in the foot muscles. Some of pathological signs were cellular degeneration, necrosis, inflammatory responses and leaky intestine (gaps) (Abd-El Azeem and Sheir, 2018). Hamed et al. (2007) found a severe vacuolization in digestive cells of *E. vermiculata* treated with molluscicidal carbamates; methiocarb and methomyl. However, the histological examination of the hepatopancreas of the treated snails showed alterations as a response to all the treatments, and revealed the degeneration of the digestive tubules and the breakdown of the basement membrane in a dose-dependent manner, leading to a severe deterioration of the tissues in the concentration of 200 mg/L thiametoxam (Ait-Hamlet et al., 2012). Hepatocyte necrosis, kidney failure, kidney cell necrosis, muscular dystrophy and anemia, heart disease could be caused by an increase in LDH in snails treated with a combination of Pirimicarb and lead acetate (Chen et al., 2011).

Conclusion

The data obtained show significant changes in the growth, biochemical parameters and enzymatic activity of the soft tissues of *E. vermiculata* snail's exposed to a fertilizer (Weafert). The fertilizer tested led to the depletion of energy reserves (lipid and carbohydrate levels) and an increase of proteins in the digestive gland of snails. An activation of the detoxification system was evidenced by an increase in the activity of GPx and LDH in the digestive gland. Lastly, structural alterations were observed in digestive gland from treated snails.

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